PCR Methodologies and Innovative Testing Platforms

The BioFire® FilmArray® Pneumonia Panel

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Panel Menu and Sample Types

15 Semiquantitative Bacteria

Bacteria

Semi-Quantitative

Acinetobacter calcoaceticus baumannii complex
Enterobacter cloacae complex
Escherichia coli
Haemophilus influenzae
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae group
Moraxella catarrhalis
Proteus spp.
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

Atypical Bacteria

Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

Viruses

8 Viruses

Adenovirus
Coronavirus
Human Rhinovirus/Enterovirus
Human Metapneumovirus
Influenza A
Influenza B
Parainfluenza Virus
Respiratory Syncytial Virus

Antimicrobial Resistance Gene

7 AMR genes

METHICILLIN RESISTANCE

mec A/C and MREJ (MRSA)
mec A/C

CARBAPENEMASES

KPC
NDM
Oxa-48-like
VIM
IMP

ESBL

CTX-M

Sample Requirements:

Sputum (including ETA) and Brochoalveolar lavage (BAL, including mini-BAL)

Rationale for Inclusion of *Legionella pneumophila* on the Pneumonia Panel

60+ named *Legionella* species, but *L. pneumophila* is the predominant pathogen of Legionnaires' disease (LD)

- > 95% infections caused by *L. pneumophila*, primarily serogroup1 (SG1)
  - Higher incidence of non-SG1 *L. pneumophila* and other *Legionella* species in nosocomial cases.

Average fatality rate ~12%, higher in elderly & immunocompromised patients (~75%)

LD not easily distinguished clinically from other types of community acquired pneumonia

- Timely diagnosis & prompt treatment cures >95% of cases
  - Special media required for culture-based diagnoses may not be ordered
  - Microscopic diagnosis difficult (<0.1% of *Legionella* cells visualized from specimens)
  - Urine antigen tests have low sensitivity (90% -40%) especially for non-SG1
- A rapid and sensitive syndromic approach for diagnosis needed
  - Few FDA-cleared commercial molecular diagnostics
    - PCR-based Curetis Unyvero™ LRT Application has a turn-around-time of ~ 6h

Edelstein PH, Lück C. Legionella. In: *Manual of Clinical Microbiology*. Vol Volume 1. 11th ed. ASM Press; 2015:887-904.
www.cdc.gov/legionella/index.html

L. pneumophila Assay Design

Assay designed to target <u>all</u> *L. pneumophila* serogroups and subspecies

- *in silico* inclusivity assessed against 196 database sequences
  - Predicted high reactivity for all 15 serogroups for which sequences are available
- *in silico* exclusivity assessed against database sequences
  - 327 sequences included 48 non-*L. pneumophila* near-neighbors
  - High specificity predicted for the assay

Validation of the *L. pneumophila* Assay: Clinical Studies

Multicenter prospective study:

- 8 geographically distinct US sites (Oct 2016 to July 2017)
- 846 BALs (including mini-BAL) and 836 sputa (both sputum and ETA)
  - ~80% hospitalized patients

Age	BAL	Sputum
0-17	5.9%	29.3%
18-65	63.9%	44.3%
>65	30.2%	26.4%

Preselected archived specimen study

- 171 frozen archived specimens (152 BAL and 19 Sputa) used for low-prevalence analytes, including *L. pneumophila*

Contrived specimen study (for extremely rare analytes)

- 1225 contrived specimens tested, including *L. pneumophila*
  - 50 samples prepared using multiple isolates, (in both BAL and Sputum)

Validation of the *L. pneumonphila* Assay: Clinical Reference Method

Comparator/Reference methods

- Atypical bacteria and viruses were compared to two conventional PCR assays followed by bidirectional sequencing.
  - **LoD of comparator assays was within 10 fold the LoD of the FilmArray assay and other commercially available assays.**

Acceptance criteria:

- A specimen was considered to be positive for an analyte if bi-directional sequencing data meeting pre-defined quality acceptance criteria matched organism-specific sequences deposited in the NCBI GenBank database (www.ncbi.nlm.nih.gov) with acceptable E-values.
- When two PCR comparator assays were used, any specimen that tested negative by both of the comparator assays was considered Negative.

Validation of the *L. pneumonphila* Assay: Clinical Performance

Study Arm	Specimen Type	Sensitivity/PPA			Specificity/PPA		
		TP/(TP +FN)	%	95% CI	TN/(TN +FP)	%	95% CI
Prospective	BAL	2/2	100	34.2-100%	833/833	100	99.5-100%
	Sputum	0/1	0	-	826/826	100	99.5-100%
Archived	BAL	1/1	100		57/57	100	93.7-100%
	Sputum						
Contrived	BAL	50/50	100	92.9-100%	599/599	100	99.4-100%
	Sputum	50/50	100	92.9-100%	521/521	100	99.3-100%

1/3 specimens positive by ref. method in prospective study was not detected by the *L. pneumonphila* assay

- Discrepancy investigation indicated sub-LoD levels of *L. pneumophila*

Only 1 archived BAL specimen could be collected

100% of contrived specimens tested at ~ 2X LoD were detected in both matrices

Validation of the *L. pneumophila* Assay: Analytical Studies : LoD and Precision

- **Limit of detection** (LoD, 60 replicates, 95% success required)
  - Dilutions of atypical bacteria spiked into contrived BAL or sputum
  - ***Legionella pneumophila* Philadelphia-1** ATCC 33152 detected at with 95% success at 5.0E+02 CFU/mL (1.6E+03 copies/mL)
- **Precision** (Reproducibility)
  - Multi-day testing at 3 sites on all platforms (30/system)
  - Tested at Negative, Low Positive (1×LoD), & Moderate Positive (3×LoD) concentrations

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result			
			FilmArray	FilmArray 2.0	FilmArray Torch	All Sites/Systems [95% CI]
			Site A	Site B	Site C	
Atypical Bacteria						
Legionella pneumophila Philadelphia-1 ATCC 33152	**Moderate Positive 3× LoD** 1.5E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** [96.0%-100%]
	Low Positive 1× LoD 5.0E+02 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** [96.0%-100%]
	None (No Analyte)	**Not Detected**	**720/720** 100%	**720/720** 100%	**720/720** 100%	**2,160/2,160** **100%** [99.8%-100%]

Validation of the *L. pneumophila* Assay: Analytical Studies : Inclusivity & Exclusivity

Analytical reactivity (inclusivity):

- Study used >350 genetically diverse isolates of viruses, bacteria, and antimicrobial resistance genes
  - Atypical bacteria and viruses were tested at concentrations within 3× LoD

Species/Subspecies	Serogroup	Source [Strain]	Test Concentration		Result
			(CFU/mL)	xLoD	
L. pneumophila	1	ATCC 33152 [Philadelphia-1]	5.0E+02	1x	*Legionella pneumophila* Detected
	3	ATCC 33155 [Bloomington-2]	1.5E+03	3x	
L. pneumophila subsp. *fraseri*	4	ATCC 33156 [Los Angeles-1]	1.5E+03	3x	
	5	ATCC 33216 [Dallas 1E]	1.5E+03	3x	
L. pneumophila subsp. *pascullei*	5	ATCC 33737 [U8W]	1.5E+03	3x	
L. pneumophila subsp. *pneumophila*	10	ATCC 43283 [Leiden 1]	1.5E+03	3x	
	14	ATCC 43703 [1169-MN-H]	1.5E+03	3x	

Analytical specificity (Cross-reactivity/exclusivity):

- 55 on-panel organisms were tested to assess the potential for intra-panel cross-reactivity
- 213 off-panel organisms included species of the same genus or otherwise genetically related to organisms & normal flora and pathogens that may be present in sputum-like and BAL-like specimens
  - 7 near-neighbor *Legionella* species also tested
  - Tested at ~100 - **100,000** fold higher than the LoD or lowest reportable level

Summary of BioFire Pneumonia Panel *L. pneumophila* assay

- Challenges posed by LD emphasize a need for a rapid and syndromic approach for future diagnostics

- The BioFire Pneumonia Panel *L. pneumophila* assay is designed to meet this need:
  - Can distinguish LD from other types of pneumonia
  - Covers all serotypes of the most common species
  - Uses multiple specimen types
  - Gives sensitive and specific results in about 1 hour after test is initiated

The BioFire Pneumonia Panel was launched in December 2018